OBSTETRICS

Modulation of vaginal immune response among pregnant women with bacterial vaginosis by *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and yeast

Sabina Cauci, PhD; Jennifer Flatow Culhane, PhD

OBJECTIVE: This study was undertaken to examine the influence of coinfections on vaginal innate and adaptive immunity, and microbial enzyme activities of pregnant women with bacterial vaginosis (BV).

STUDY DESIGN: The population consisted of 265 singleton pregnant women in early gestation (<20 weeks) with BV (Nugent 7-10) who had vaginal fluid collected for measurement of interleukin-1β (IL-1β) and IL-8 concentrations, number of neutrophils, immunoglobulin A against *Gardnerella vaginalis* (anti-Gvh IgA), and activities of microbial sialidase and prolidase.

RESULTS: Among women with BV, median levels of vaginal IL-1β (4-fold, $P = .005$), IL-8 (4-fold, $P < .001$), and neutrophils (6-fold, $P = .013$) were greatly increased in women with *T vaginalis* with respect to women without any coinfection. Yeast increased the level of IL-8 (5-fold, $P < .001$), but not IL-1β ($P = .239$) and neutrophils ($P = .060$). *Chlamydia trachomatis* and *Neisseria gonorrhoeae* had no effect on vaginal cytokines. None of the coinfections influenced vaginal anti-Gvh IgA, sialidase and prolidase activities.

CONCLUSION: The strong proinflammatory cytokine induction by *T vaginalis* may contribute to the observed increase in preterm birth among BV positive women coinfected with *T vaginalis* treated with metronidazole.

Key words: adverse pregnancy outcome, bacterial vaginosis, interleukin-1β, interleukin-8, prolidase, sialidase, Trichomonas vaginalis, vaginal neutrophils

Cite this article as: Cauci S, Flatow Culhane J. Modulation of vaginal immune response among pregnant women with bacterial vaginosis by Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, and yeast. Am J Obstet Gynecol 2007;196;133.e1-133.e7.

Evidence suggests that the profile of vaginal immune and microbial biomarkers has an impact on adverse pregnancy outcomes related to infection.[1–4] Innate immunity is considered the first line of mucosal defense against microbes, in part via the induction of an array of proinflammatory cytokines and antimicrobial factors, and in part by stimulation of the adaptive immunologic response. However, many features of the innate vaginal response to microbes are not yet understood.[1,4] Improved understanding of the regulation of local immunity could provide insight into the nature of the relationship between vaginal infections and adverse birth outcomes,[1] and/or human immunodeficiency virus (HIV) acquisition and transmission.[5,6]

★ EDITORS' CHOICE ★

Vaginal infections such as bacterial vaginosis (BV) and *Trichomonas vaginalis* in pregnant women have been associated with preterm birth.[7–12] However, the pathophysiology underpinning these relationships is not known. Recent studies have demonstrated that treatment of BV in pregnancy with antibiotics clears the infection but does not reduce the risk of preterm birth or the incidence of associated adverse outcomes.[13–15] In a study with alarming results, metronidazole treatment in women with *T vaginalis* reduced the infection rate but increased the incidence of preterm birth.[16] Understanding the alterations in vaginal mucosal immune profiles associated with BV and *T vaginalis* coinfection may help to elucidate the confusing results of antibiotic treatment.[12]

BV is a complex polymicrobial disorder characterized by depletion of lactobacilli and overgrowth of a mixed variable anaerobic and facultative flora, including *Gardnerella vaginalis, Prevotella* spp, *Bacteroides* spp, *Mobiluncus* spp, gram-positive cocci, and genital mycoplasma. Sialidases and prolidases are enzymes produced by anaerobic bacteria likely involved in the pathogenesis of BV.[17,18] The action of these hydrolytic enzymes can potentially alter the immune signals and promote the degradation of host factors.[17–19] A recent study on Danish pregnant women showed that high sialidase and/or prolidase activity combined with elevated vaginal pH 5 or

From the Department of Biomedical Sciences and Technologies, School of Medicine, University of Udine, Italy (Dr Cauci); and the Department of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA (Dr Culhane).

Part of the data were presented in preliminary form at the 25th Annual Meeting of the Society for Maternal-Fetal Medicine, February 7-12, 2005, Reno, NV.

Reprint requests: Sabina Cauci, PhD, Dipartimento di Scienze e Tecnologie Biomediche, Facoltá di Medicina e Chirurgia, Piazzale Kolbe 4, 33100 Udine, Italy.scauci@mail.dstb.uniud.it

This study was supported by Centers for Disease Control and Prevention/ATPM TS-0626 and TS-0561.

0002-9378/free

doi: 10.1016/j.ajog.2006.08.033

greater, are strong risk factors for early preterm birth (<32 weeks of gestation), low birthweight (LBW), and very LBW (<1500 g at birth).[20] In the same population, high levels of specific immunoglobulin A (IgA) against the toxin produced by *G vaginalis* (anti-Gvh IgA) were protective for adverse pregnancy outcomes.[21]

BV is not characterized by an increase of vaginal polymorphonuclear neutrophils (PMN), unlike trichomoniasis, which usually provokes a large increase of PMN.[11] However, BV and trichomoniasis are both characterized by an elevated vaginal pH (>4.5), and malodorous discharge. Frequently, women with BV also have other vaginal infections, especially *T vaginalis, C trachomatis,* and *N gonorrhoeae*.[12,22,23] A recent study showed that BV was positively associated with trichomoniasis with odds ratio (OR) = 5.93 (95% CI = 1.34-26.24), and *Chlamydia* and/or gonorrhea with OR = 8.87 (95% CI = 1.21-65.09).[23]

Increasing evidence points to interleukin (IL)-1β as a crucial cytokine, which may mediate reproductive tract infection-related adverse outcomes.[1,2,24,25] Genc et al[25] proposed an exaggerated vaginal IL-1β response, possibly linked to genetic polymorphisms, as a risk factor for adverse pregnancy outcome. IL-1β is a master proinflammatory cytokine responsible for a cascade of innate and adaptive responses.[26] IL-1β rises in response to several stimuli, including a vast array of pathogens. Increased vaginal concentrations of IL-1β have been consistently found in women with BV, and HIV.[27–30] In vaginal fluid, IL-1β was positively associated with IL-8 (CXCL8) a potent chemokine.[29] Both of these proinflammatory cytokines were consistently correlated with the number of vaginal neutrophils, in healthy and BV-positive women.[4,27,29] Though a number of studies have examined vaginal immunity in BV positive women, few investigations have examined the modulation of vaginal immunity by *T vaginalis* in BV-positive women. A study showed that vaginal discharges of symptomatic trichomoniasis patients had large amounts of IL-8.[31] However, to our knowledge, there are no data on the effects of *T vaginalis* on vaginal levels of IL-1β in pregnant women with BV.

In this investigation, we explored the role of concurrent vaginal infections, such as *T vaginalis, C trachomatis, N gonorrhoeae,* and yeast on vaginal concentrations of IL-1β and IL-8, number of neutrophils, anti-Gvh IgA, and sialidase and prolidase activities in pregnant women with BV.

Materials and Methods

All women enrolling for prenatal care at three hospital-based clinics and 1 community-based clinic between Jan. 1, 2002, and Sept. 7, 2004, were screened for eligibility into the study. Women were considered eligible for participation if they: (1) were less than 20 weeks pregnant at the time of enrollment, (2) spoke either English or Spanish, (3) had a singleton intrauterine pregnancy, (4) were not HIV positive, (5) did not seek a therapeutic abortion, (6) were 18 years of age or older, (7) underwent a routine pelvic examination at the enrollment visit, and (8) had no vaginal bleedings at the time of recruitment.

At the time of enrollment, participants were interviewed by trained female interviewers to ascertain a wide array of demographic, behavioral, psychosocial, and medical history data. In addition to this self-reported information, vaginal secretions were obtained from the posterior wall of the vaginal fornix at the time of routine speculum examination. Of the 7 swabs collected, 5 were inoculated into sterile saline solution and immediately snap frozen in liquid nitrogen. Two swabs were used to create air-dried vaginal smears. Air-dried vaginal smears were stained and assessed for BV according to Nugent criteria.[32] A score of 0-3 was considered normal, a score of 4-6 corresponded to intermediate BV status, and a score of 7-10 was defined as positive for BV. Neutrophils and clue cells were determined on the Gram-stained smear at 1000× magnification in 5 nonadjacent fields. For statistical calculations, number of neutrophils was set equal to 40 when more than 30, and percent of clue cells was set equal to 30 when more than 20%.[28,29] Neutrophil counts were not available for 2 women in this study. After assessing the BV status of participants, a random sample of 265 BV-positive women was chosen. For these women, we retrieved and analyzed vaginal fluid from 5 thawed swabs. Samples were centrifuged at 700*g* for 3 minutes at 4°C and the supernatant was aliquoted and immediately frozen and stored at −80°C.

BV-positive women were further divided into mutually exclusive groups according to the presence of concurrent vaginal infections. *T vaginalis* was evaluated by Xenostrip examination (MP Biomedicals LLC, Burlingame CA). Diagnosis of *N gonorrhoeae*, and *C trachomatis* was based on urine nucleic acid amplification by the APTIMA Combo 2 Assay (Gen-Probe, Inc, San Diego, CA), which is a nucleic acid probe using target capture for ribosomal RNA. Yeast vaginal colonization was determined by presence of hyphae and/or yeast spores in the Gram-stained smear.

IL-1β (Sanquin, Amsterdam, The Netherlands) and IL-8 (Bender Med Systems, San Bruno, CA) were quantified in the vaginal fluid by commercial enzyme-linked immunosorbent assay (ELISA) kits and measurements were performed according to the manufacturer's instructions.[29] The intra- and interassay coefficient variations for both assays were less than 10%. The lower detection limit was 1 pg/mL for human IL-1β, and 8 pg/mL for IL-8. Fifteen women had undetectable IL-1β, and 2 women had undetectable IL-8 levels. Values greater than the 75th percentile were considered high levels of ILs, those below the 25th percentile were considered low levels, and values between the 25th and the 75th percentile were considered medium levels.

Sialidase activity was determined by incubation of 50 μL of the vaginal sample with the specific substrate at pH 5.0, as described previously.[21] Sialidase activity was expressed as nanomoles of converted substrate (methoxyphenol) produced by comparison with a standard curve of pure methoxyphenol. Prolidase activity was determined as previously described by use of a chromogenic substrate.[21] Absorbance in milliopitcal density units (mOD) was read at 405 nm.

Values of anti-Gvh IgA were evaluated by ELISA in mOD units read at 405 nm as described.[21]

All measurements were performed in duplicate.

Statistical analysis

Cytokine concentrations were not normally distributed, thus the Mann-Whitney *U* test was used to compare vaginal factor levels between BV subgroups. Difference of proportions was assessed by a 2-tailed Fisher's χ^2 exact test. The Kruskal-Wallis test was used to compare 3 or more groups. Any *P* value less than .05 was considered statistically significant. The software package SPSS (Statistical Package for Social Sciences, Chicago, IL) was used for data analyses.

RESULTS

Demographic characteristics, number (percent) or mean ± SD values, of the entire cohort of 265 pregnant women with BV are shown in Table 1. The majority of participants were black, single, considered low income, and at enrollment, were in early gestation (mean 11.7 ± 3.59 weeks of gestation).

Two hundred (200/265 = 75.5%) women had BV without any coinfection (BV controls). Only 1 woman had syphilis and was excluded from further analyses, leaving a total cohort of 264 BV-positive women. Of the remaining 64 BV-positive women, 26 were coinfected with yeast, 12 were coinfected with *T vaginalis*, 9 with *C trachomatis*, and 1 with *N gonorrhoeae*. In addition, 6 BV-positive women were coinfected with both *T vaginalis* and *C trachomatis*, 5 with *C trachomatis* and *N gonorrhoeae*, 2 with yeast and *T vaginalis*, 1 with *T vaginalis* and *N gonorrhoeae*, 1 with *N gonorrhoeae* and yeast, and 1 with *C trachomatis* and yeast. Thus, a total of 21 women had *vaginalis* (21/264 = 8.0%), 21 women had *C trachomatis* (21/264 = 8.0%), 8 women had *N gonorrhoeae* (8/264 = 3.0%) and, 30 women had yeast (30/264 =11.4%).

Table 2 shows levels of vaginal biomarkers (median and interquartile values) for the entire cohort. Wide ranges for vaginal biomarkers were observed. In addition, Table 2 compares vaginal biomarker values between BV controls and women with only 1 coinfection, with the exception of the 1 woman coinfected with *N gonorrhoeae*. Of note, women with BV and *T vaginalis* had much higher concentrations of IL-1β (4.2-fold, $P = .005$), IL-8 (4.1-fold, $P < .001$), and neutrophils (6-fold, $P = .013$) compared with those with BV alone. However, *T vaginalis* had no influence on sialidase and prolidase activities or anti-Gvh IgA levels. On the contrary, the percent of clue cells was lower in the BV-positive *T vaginalis* group when compared with the BV-positive control group. The BV-positive *C trachomatis* group was not different from controls for any vaginal biomarker. Interestingly, additional colonization with yeast had no influence on IL-1β levels in BV-positive women. However, yeast induced a large increase of the chemokine IL-8 (5.3-fold, $P = .001$), and tended to increase neutrophils (2.2-fold, $P = .060$) with respect to BV controls. Yeast coinfection had no effect on sialidase, prolidase, or anti-Gvh IgA levels. The BV-positive yeast positive group had a slightly lower number of clue cells compared with BV controls.

By using the IL-1β values obtained from the entire study sample (n = 264), we constructed cut points for 3 categories; low (< 25th percentile, < 36 pg/mL), medium (from the 25th to the 75th percentile, 36-399 pg/mL), and high (> 75th percentile, > 399 pg/mL) levels of IL-1β (Table 3). *T vaginalis* was more

TABLE 1
Demographic characteristics of the 265 BV-positive pregnant women enrolled

Characteristic	Frequency or mean ± SD or %
Black (non-Hispanic)	73.2%
Hispanic	21.4%
White (non-Hispanic)	4.3%
Others	1.2%
Nulliparity	40.5%
Posthigh school education	22.0%
High school completed	44.8%
High school not completed	33.2%
Single	75.7%
Maternal age (y)	24.2 ± 5.18
Gestation (wk)	11.7 ± 3.59
Yearly income (US $)	11822 ± 11305
Smoking	32.4%
Ever smoked 20 or more cigarette per week among smokers	35.1%
Number of cigarettes per week before pregnancy among smokers	23.5 ± 48.59
Douching in the year before pregnancy	51.5%
Frequency of douching per week	0.20 ± 0.44
Lifetime sexual partners	6.3 ± 8.37
7 or more sexual partners lifetime	25.1%
T vaginalis	8.3%
C trachomatis	7.9%
N gonorrhoeae	3.0%
Yeast	11.3%
Syphilis	0.38%

TABLE 2
Comparison of vaginal biomarkers between pregnant women positive for BV only (BV controls) and those positive for BV and 1 additional coinfection

Vaginal biomarker	All BV-positive women (n = 264)	BV-positive controls (n = 200)	TV^+ coinfection (n = 12)	P*	CT^+ coinfection (n = 9)	P*	$Yeast^+$ coinfection (n = 26)	P*	P†
IL-1beta (pg/mL)	153 (36-399)	125 (26-338)	527 (160-1229)	.005	83 (60-348)	.852	207 (38-503)	.239	.033
IL-8 (pg/mL)	2357 (680-6994)	1775 (599-5220)	7349 (3655-18433)	< .001	2055 (564-6559)	.748	9391 (1293-20689)	.001	< .001
Neutrophils number	3 (0-10)	2.0 (0.0-7.0)	12.0 (4.25-26.7)	.013	3.0 (0.5-15)	.554	4.5 (1.0-18)	.060	.027
Sialidase (nmol substrate)	3.00 (1.01-6.12)	3.15 (1.05-6.33)	2.14 (0.33-4.28)	.180	2.06 (0.79-4.90)	.455	2.46 (0.39-7.73)	.320	.400
Prolidase (mOD)	558 (216-1422)	566 (226-1377)	429 (87-2661)	.848	640 (96-2404)	.780	459 (101-1003)	.213	.657
% clue cells	30 (10-30)	30 (13-30)	0 (0-9)	< .001	22 (13-30)	.557	30 (0-30)	.037	< .001
Anti-Gvh IgA (mOD)	82 (16-209)	84 (14-196)	82 (44-240)	.667	100 (0-160)	.771	55 (29-213)	.859	.954

Median (interquartile) values are reported.
TV^+, *T vaginalis* positive, no other coinfection; CT^+, *C trachomatis* positive, no other coinfection; $yeast^+$, yeast positive, no other coinfection.
* Mann-Whitney *P* values were calculated in respect to the control group (n = 200) without any vaginal infection except BV.
† Kruskal-Wallis *P* values applied to the 4 groups: BV controls, TV^+, CT^+, and $yeast^+$ groups.

frequently found in the high IL-1β group (14/65 = 21.5%) than in the medium (6/135 = 4.4%) and low IL-1β (1/64 = 1.6%) groups, $P < .001$. Eight *C trachomatis*-positive women had high IL-1β levels (8/65 = 12.3%), but 5 of them were also *T vaginalis* positive. The frequency of *N gonorrhoeae* and yeast was not different in the IL-1β subgroups.

We further examined the frequency of coinfections according to progressive levels of vaginal IL-8 in the 264 pregnant women with BV by using a similar methodology. Table 4 compares women with low (< 25th percentile, < 680 pg/mL), medium (from the 25th to the 75th percentile, 680-6994 pg/mL), and high (> 75th percentile, > 6994 pg/mL) levels of IL-8. Approximately 20% of women with high levels of IL-8 were *T vaginalis* positive vs 0% of women in the lower IL-8 group ($P < .001$). In addition, 24% of women with high levels of IL-8 were yeast positive vs 6.1% in the medium IL-8 group ($P < .001$), and 9.1% in the lower IL-8 group ($P = .034$).

Finally, Table 5 shows the frequency of coinfections in subgroups of women with increasing levels of vaginal neutrophils. The only coinfection significantly more frequent in the high neutrophil group was *T vaginalis.*

Comment

Until recently, most studies describing the association between BV and adverse outcomes have not explored the host/microbial interactions at the level of the vaginal mucosa.[1,2,4] To our knowledge, this study is the largest investigation of modulation of vaginal immunity in pregnant women with BV with and without concurrent infections. In addition to women exclusively infected with BV, we focused on those coinfected with *T vaginalis*, *C trachomatis, N gonorrhoeae,* and yeast.

This study focused on vaginal IL-1β levels as this cytokine is one of the main proinflammatory factors able to activate innate and adaptive immunity against microbes. The "downstream" effects of an IL-1β induced cascade include activation of white cells to kill microorganisms and should therefore be beneficial to the

TABLE 3
Frequency of coinfections in 264 women with BV according to IL-1β levels in vaginal fluid

Coinfection	Low IL-1β (n = 64)	P*	Medium IL-1β (n = 135)	High IL-1β (n = 65)	P†	P‡	P§
T vaginalis	1 (1.6%)	.433	6 (4.4%)	14 (21.5%)	< .001	< .001	< .001
C trachomatis	1 (1.6%)	.065	12 (8.9%)	8 (12.3%)	.459	.033	.067
N gonorrhoeae	1 (1.6%)	.433	6 (4.4%)	1 (1.5%)	.431	> .99	.392
Yeast	6 (9.4%)	> .99	13 (9.6%)	11 (16.9%)	.164	.298	.267

Low (<25th percentile,< 36 pg/mL), medium (25th-75th percentile, 36-399 pg/mL), and high (>75th percentile, > 399 pg/mL) values of vaginal IL-1β concentrations were compared.

* 2-sided Fisher's exact test comparing low vs medium IL-1β groups.

† 2-sided Fisher's exact test comparing medium vs high IL-1 β groups.

‡ 2-sided Fisher's exact test comparing low vs high IL-1 β groups.

§ Kruskal-Wallis test applied to the 3 groups.

host.[26] However, if the immune response is exaggerated, elevated levels of IL-1β could be harmful to the host through induction of an excessive inflammatory reaction.[1,2,25] Thus, the margin between beneficial and adverse effects of IL-1β may be fairly narrow. Different signals by many different pathogens can trigger a rise of this pleiotropic cytokine. In our study we found a wide range of IL-1β concentrations (0-7125 pg/mL) in vaginal fluid of 264 pregnant women with BV with and without coinfections. Concurrent infection with *T vaginalis* was able to modulate IL-1β levels in BV-positive women. *T vaginalis* was a powerful inducer of IL-1β, increasing median levels 4-fold with respect to BV-positive women without any concurrent infection. This is not surprising, given that *T vaginalis* is a protozoa known to induce a strong inflammatory response in vaginal mucosa.[4,11,31] However, the actual levels of vaginal proinflammatory cytokines in pregnant women with BV and *T vaginalis* have never been reported before.

It is worth mentioning that around 20% of BV-positive women with high levels of IL-1β in our sample were also infected with *T vaginalis.* This suggests that the proportion of women with BV and high IL-1β levels may vary according to the prevalence of concurrent infection with *T vaginalis* in the study population; a finding that has serious implications for studies comparing US pregnant women and women from other locations with lower rates of trichomoniasis, for example Western European samples.[12] The difference in coinfection rates with *T vaginalis* among BV-positive women may account for the lack of association between BV and adverse pregnancy outcomes in studies conducted on European population.[21] In addition, the success of antibiotic treatment in BV-positive women found in some European studies[14,15] may be attributable to a lower rate of coinfection with other sexually transmitted infections (STIs). Even within geographically similar populations, demographic characteristics like race may impact the link between BV and reproductive outcomes through differential rates of coinfection with STIs leading to alterations in vaginal immunity.[1,4,33] For example, a large study of midgestation pregnant American women showed race-specific prevalence rate of trichomoniasis: 22.8% for black; 6.6% for Hispanic and 6.1% for white women.[33] A weakness of our study to assess *T vaginalis* prevalence was the use of the XenoStrip rapid test that has a sensitivity of 77-90% and a specificity of 93-99% when compared with culture.[34]

In our study, *C trachomatis* had no effect on vaginal proinflammatory cytokines and neutrophils. These findings are in line with a recent study performed in nonpregnant women demonstrating

TABLE 4
Frequency of coinfections in 264 women with BV according to IL-8 levels in vaginal fluid

Coinfection	Low IL-8 (n = 66)	P*	Medium IL-8 (n = 132)	High IL-8 (n = 66)	P†	P‡	P§
T vaginalis	0 (0%)	.054	8 (6.1%)	13 (19.7%)	.003	<.001	<.001
C trachomatis	5 (7.6%)	.763	8 (6.1%)	8 (12.1%)	.169	.561	.330
N gonorrhoeae	3 (4.6%)	.402	3 (2.3%)	2 (3.0%)	>.99	>.99	.680
Yeast	6 (9.1%)	.557	8 (6.1%)	16 (24.2%)	<.001	.034	.001

Low (<25th percentile, < 680 pg/mL), medium (25th-75th percentile, 680-6994 pg/mL), and high (>75th percentile, > 6994 pg/mL) values of vaginal IL-1β concentrations were compared.

* 2-sided Fisher's exact test comparing low vs medium IL-8 groups.

† 2-sided Fisher's exact test comparing medium vs high IL-8 groups.

‡ 2-sided Fisher's exact test comparing low vs high IL-8 groups.

§ Kruskal-Wallis test applied to the 3 groups.

TABLE 5
Frequency of coinfections in 264 women with BV according to neutrophils levels in vaginal fluid determined on the Gram-stained vaginal smear

Coinfection	Low neutrophils (n = 68)	P*	Medium neutrophils (n = 126)	High neutrophils (n = 68)	P†	P‡	P§
T vaginalis	2 (2.9%)	.498	7 (5.6%)	12 (17.6%)	.010	.009	.003
C trachomatis	3 (4.4%)	.266	12 (9.5%)	6 (8.8%)	> .99	.493	.440
N gonorrhoeae	2 (2.9%)	>.99	4 (3.2%)	2 (2.9%)	> .99	> .99	.994
Yeast	5 (7.4%)	.459	15 (11.9%)	10 (14.7%)	.655	.273	.395

Low (<25th percentile, 0 number), medium (25th-75th percentile, 0-10 number), and high (>75th percentile, > 10 number) values of vaginal neutrophils were compared.

* 2-sided Fisher's exact test comparing low vs medium neutrophils groups.

† 2-sided Fisher's exact test comparing medium vs high neutrophils groups.

‡ 2-sided Fisher's exact test comparing low vs high neutrophils groups.

§ Kruskal-Wallis test applied to the 3 groups.

that IL-1 and IL-8 levels were not different in cervical secretions of *C trachomatis* positive compared with uninfected control women.[35] We did not observe an increased proinflammatory response in vaginal fluid associated with BV and *N gonorrhoeae* infection. Our results concur with observations of other authors about *N gonorrhoeae* infection at vaginal level.[4,36] Interestingly, we found that vaginal yeast colonization did not influence IL-1β, significantly increased IL-8 concentrations, and only marginally increased neutrophils. Possibly, augmentation of vaginal cytokines in response to yeast infection involves a pattern of host white cells different from that involved in nonfungal infections, ie, including mainly mast cells, which when stimulated produce large amounts of IL-8.[4,11,37,38] Because yeast colonization is not generally considered a risk factor for adverse pregnancy outcomes, it is tempting to speculate that an increase in vaginal IL-1β, more than an increase in IL-8 per se, confers risk in pregnant women. A limitation of our study consisted in evaluation of yeast colonization by Gram stain, which has a reduced sensitivity compared with yeast culture methods.[11]

None of the concurrent infections studied modulated the levels of sialidase and prolidase activities in women with BV. This observation is consistent with the fact that *T vaginalis, C trachomatis, N gonorrhoeae* and yeast are not known to produce these specific enzymes, which are peculiar to anaerobic bacteria present in the BV milieu. In addition, it is of note that the vaginal-specific IgA response (anti-Gvh IgA) in BV-positive women was not modulated by any coinfection. Anti-Gvh IgA response is considered protective against adverse pregnancy outcomes,[21] thus it appears that coinfections are not involved in dampening this adaptive response in BV-positive pregnant women, this observation could be important in future immune modulation strategies aimed to increase host defenses.

An excessive or unbalanced release of proinflammatory factors may alter the mucosal balance between tissue destruction and repair and be linked to enhanced penetration and replication of bacterial/viral pathogens. High levels of IL-1β have been suggested as a risk factor for preterm birth.[25] Our current results show that induction of high levels of vaginal IL-1β can derive from different microbial signals. In our population of mainly black women at 12 weeks of gestation with BV, 21.5% of high IL-1β responders were also *T vaginalis* positive. Further studies should assess if women with elevated vaginal IL-1β with or without *T vaginalis* infection are at increased risk of preterm birth.

BV in pregnancy has been consistently associated with several adverse outcomes, including spontaneous preterm delivery, and LBW.[7–9] In addition, BV has been associated with upper genital tract infections, urinary infections, and increased risk of sexual acquisition of HIV and herpes simplex virus type 2 (HSV-2) infection.[4,23] The association of trichomoniasis with the above mentioned adverse outcomes is much more controversial.[12] Even weaker are associations of *C trachomatis*[39] and yeast infections with adverse pregnancy outcomes.[4] This suggests that a large induction of vaginal IL-1β and/or IL-8 may not necessarily be a risk factor for adverse outcome unless such increase is accompanied by other (largely uncharacterized) negative events induced by microorganisms and/or determined by the host background.

In summary, the overall scenario in BV is multifaced. Our results indicate that the presence of concurrent infections may produce different immune profiles in BV-positive women. It was previously known that BV induces high levels of vaginal IL-1β in comparison to healthy women,[2] but it was not estimated to what extent concurrent infections modulate levels of IL-1β in BV positive women. We first determined that among the infections commonly found in parallel with BV, only *T vaginalis* affected IL-1β. On the other hand, vaginal levels of IL-8 were positively modulated by both *T vaginalis* and yeast, 2 vaginal syndromes known to cause clinically evident vaginal inflammatory signs and symptoms with leukocyte accumulation in vaginal secretions.

Our hypothesis is that the combination of specific immune/enzyme/microbial factors can determine the final risk for adverse outcome among BV-positive women. Beneficial/adverse effects of

therapeutic interventions could be associated with the microbial/immunity profile of a given BV-positive pregnant woman. We cannot assess this issue on the population presently examined as this study was not designed to assess treatment of BV. If our hypothesis is supported by further evidence, the immune/enzyme/microbial profile should be taken into account when developing intervention trials. Finally, it seems advisable that future therapeutic treatments aim to modulate local immunity in addition to eradicating microorganisms.[40] ■

ACKNOWLEDGMENTS

We thank Dr Giorgia Casabellata, Department of Biomedical Sciences and Technologies, University of Udine, Udine, Italy, for slide readings.

REFERENCES

1. Romero R, Chaiworapongsa T, Kuivaniemi H, at al. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. Am J Obstet Gynecol 2004;190: 1509-19.

2. Cauci S. Vaginal immunity in bacterial vaginosis. Curr Infect Dis Rep 2004;6:450-6.

3. Culhane JF, De Santo D, Goldenberg RL, et al. Variation in Nugent score and leukocyte count in fluid collected from different vaginal sites. Obstet Gynecol 2005;105:120-3.

4. Russell MW, Sparling PF, Morrison RP, et al. Mucosal immunology of sexually transmitted diseases. In: Mestecky J, Lamm ME, McGhee JR, Bienenstock J, Mayer L, Strober W, (eds). Mucosal immunology. 3rd ed. Boston: Elsevier Academic Press; 2005:1693-720.

5. Schwebbe JR. Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually transmitted diseases. J Infect Dis 2005;192:1315-7.

6. Chi BH, Mudenda V, Levy J, et al. Acute and chronic chorioamnionitis and the risk of perinatal human immunodeficiency virus-1 transmission. Am J Obstet Gynecol 2006;194:174-81.

7. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. N Engl J Med 1995;333:1737-42.

8. McGregor JA, French JI. Bacterial vaginosis in pregnancy. Obstet Gynecol Surv 2000;55: S1-19.

9. Goldenberg RL, Culhane J. Infection as a cause of preterm birth. Clin Perinatol 2003;30: 677-700.

10. Cotch MF, Pastorek JG, Nugent RP, et al. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. Sex Transm Dis 1997;24:353-60.

11. Sobel JD. Vaginitis. N Eng J Med 1997; 26:1896-903.

12. Sobel JD. What's new in bacterial vaginosis and trichomoniasis? Infect Dis Clin North Am 2005;19:387-406.

13. Carey JC, Klebanoff MA, Hauth JC, et al. Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N Engl J Med 2000;342: 534-40.

14. Riggs MA, Klebanoff MA. Treatment of vaginal infections to prevent preterm birth: a meta-analysis. Clin Obstet Gynecol 2004;47:796-807.

15. Okun N, Gronau KA, Hannah ME. Antibiotics for bacterial vaginosis or *Trichomonas vaginalis* in pregnancy: a systematic review. Obstet Gynecol 2005;105:857-68.

16. Klebanoff MA, Carey JC, Hauth JC, et al. Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. N Engl J Med 2001;345:487-93.

17. Cauci S, Driussi S, Monte R, et al. Immunoglobulin A response against *Gardnerella vaginalis* hemolysin and sialidase activity in bacterial vaginosis. Am J Obstet Gynecol 1998;178: 511-5.

18. Cauci S, Monte R, Driussi S, et al. Impairment of the mucosal immune system: IgA and IgM cleavage detected in vaginal washings of a subgroup of patients with bacterial vaginosis. J Infect Dis 1998;178:1698-706.

19. Cauci S, Guaschino S, Driussi S, et al. Correlation of local interleukin-8 with immunoglobulin A against *Gardnerella vaginalis* hemolysin and with prolidase and sialidase levels in women with bacterial vaginosis. J Infect Dis 2002;185:1614-20.

20. Cauci S, McGregor J, Thorsen P, et al. Combination of vaginal pH with vaginal sialidase and prolidase activities for prediction of low birth weight and preterm birth. Am J Obstet Gynecol 2005;192:489-96.

21. Cauci S, Thorsen P, Schendel DE, et al. Determination of immunoglobulin A against *Gardnerella vaginalis* hemolysin, sialidase, and prolidase activities in vaginal fluid: implications for adverse pregnancy outcomes. J Clin Microbiol 2003;41:435-8.

22. Wiesenfeld HC, Hillier SL, Krohn MA, et al. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. Clin Infect Dis 2003;36:663-8.

23. Myer L, Denny L, Telerant R, et al. Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. J Infect Dis 2005;192:1372-80.

24. Romero R, Mazor M, Brandt F, et al. Interleukin-1α and interleukin-β in preterm and term parturition. Am J Reprod Immunol 1992;27: 117-23.

25. Genc MR, Witkin SS, Delaney ML, et al. A disproportionate increase in IL-1β over IL-1ra in the cervicovaginal secretions of pregnant women with altered vaginal microflora correlates with preterm birth. Am J Obstet Gynecol 2004;190:1191-7.

26. Dinarello CA. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol 2002;20: S1-13.

27. Sturm-Ramirez K, Gaye-Diallo A, Eisen G, et al.. High levels of tumor necrosis factor-alpha and interleukin-1beta in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. J Infect Dis 2000;182:467-73.

28. Cauci S, Driussi S, Guaschino S, et al. Correlation of local interleukin-1beta levels with specific IgA response against *Gardnerella vaginalis* cytolysin in women with bacterial vaginosis. Am J Reprod Immunol 2002;47:257-64.

29. Cauci S, Guaschino S, de Aloysio D, et al. Interrelationships of interleukin-8 with interleukin-1beta and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women. Mol Hum Reprod 2003;9:53-8.

30. Zara F, Nappi RE, Brerra R, et al. Markers of local immunity in cervico-vaginal secretions of HIV infected women: implications for HIV shedding. Sex Transm Infect 2004;80:108-12.

31. Shaio MF, Lin PR. Leucotriene B4 levels in the vaginal discharges from cases of trichomoniasis. Ann Trop Med Parasitol 1995;89:85-8.

32. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol 1991;29:297-301.

33. Goldenberg RL, Klebanoff MA, Nugent R, et al. Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1996;174:1618-21.

34. Gaydos CA. Rapid tests for sexually transmitted diseases. Curr Infect Dis Rep 2006; 8:115-24.

35. Reddy BS, Rastogi S, Das B, et al. Cytokine expression pattern in the genital tract of *Chlamydia trachomatis* positive infertile women – implication for T-cell responses. Clin Exp Immunol 2004;137:552-8.

36. Hedges SR, Sibley DA, Mayo MS, et al. Cytokine and antibody response in women infected with *Neisseria gonorrhoeae*: effects of concomitant infections. J Infect Dis 1998;178: 742-51.

37. Fidel PL. Immune regulation and its role in the pathogenesis of Candida vaginitis. Curr Infect Dis Rep 2003;5:488-93.

38. Hundley TR, Gilfillan AM, Tkaczyk C, et al. Kit and FcεRI mediate unique and convergent signals for release of inflammatory mediators from human mast cells. Blood 2004;104: 2410-7.

39. Andrews WW, Klebanoff MA, Thom EA, et al. Midpregnancy genitourinary tract infection with *Chlamydia trachomatis*: association with subsequent preterm delivery in women with bacterial vaginosis and *Trichomonas vaginalis*. Am J Obstet Gynecol 2006;194:493-500.

40. Carey JC, Klebanoff MA. Is a change in the vaginal flora associated with an increased risk of preterm birth? Am J Obstet Gynecol 2005; 192:1341-7.